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SOME OBSERVATIONS ON THE AXENIC GROWTH OF *TETRAHYMENA PYRIFORMIS* MC[1]

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ABSTRACT

Tetrahymena pyriformis MC was tested for growth in media containing different carbohydrate and nitrogen sources. The ciliates achieved greater growth rate in carbohydrate supplemented casein media than the unsupplemented casein medium, but did not show significant difference to the effect of glucose or starch. In media of lower nitrogen contents (<1 mg-nitrogen/ml), the level of carbohydrate supplementation did not affect the growth appreciably. At higher nitrogen levels, greater growth rate was obtained as carbohydrate level was increased; however, with carbohydrate content higher than 0.3%, very slight change of growth rate was observed.

The growth of the ciliates on various nitrogen sources showed no correlation with the nutritive values of the proteins. They failed to grow in media containing amino acids or gelatin as the nitrogen source in 4-day incubation period, but growth was observed after prolonged incubation. Media containing Tween 80 or sterols retarded growth of the ciliates. The effect of nitrogen source on metabolic changes appeared to be a major determinant of growth rather than osmotic effect.

Earlier studies on *Tetrahymena pyriformis* revealed that the unicellular organism bears some similarity to vertebrates in the requirement of essential amino acids for maintaining normal growth. Utilizing the ciliates, Rosen and Fernell (1, 2), Rosen, Scott and Smith (3) and Teunisson (4) determined the relative nutritive values of intact proteins and protein concentrates. The advantage of using this organism for protein assays was discussed by Kidder and Dewey (5) and the microbiological assay of protein quality was reviewed by Rosen (6).

In view of the simplicity of the microbiological assay and the small amount of material required in adopting the ciliates as the test organism, effort has been made in the present experiments on the feasibility of using a native strain for such purpose. Axenic growth of the ciliates in media containing different carbohydrate and nitrogen sources was also observed for metabolic and nutritional studies on this organism.

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MATERIAS AND METHODS

Test Organism: Tetrahymena pyriformis MC, was isolated locally and maintained under axenic condition in this laboratory. Stock cultures were kept at 25 C in cotton plugged 250 ml Erlenmeyer flasks containing 50 ml of the nutrient broth of the following composition (% w/v): peptone 2, yeast extract 0.1, glucose 0.5 and sodium chloride 0.1; adjusted to pH 7.1. The cultures were transferred every fortnight and checked periodically for bacteria contamination.

Inoculum: Prosperously grown, 3-day peptone cultures of the cliates were used as inocula. The ciliates were concentrated by centrifugation and washed by sterilized distilled water under aseptical condition. The washed ciliates were resuspended in water to the original concentration. For inoculation, each tube or flask received an inoculum of two drops of the washed ciliate suspension.

Basal medium for experimental cultures: The basal medium (TABLE I) essentially similar to that of Kidder and Dewey (5) and Teunisson (4) was used in the growth experiments. Energy source, minerals, pyrimidine and purine bases, and nitrogen sources were dissolved separately in appropriate concentrations of ten fold the final concentration adjusted to pH 7.2. Solutions were mixed and sterilized in foam plugged 50 ml Erlenmeyer flasks or in 25×200 mm test tubes at 120 C for 15 minutes. Vitamins were dissolved separately to form a 10× the final concentration with pH adjusted to 7.2 and sterilized by filtering through a Seitz filter. One ml of the sterilized vitamin solution was added aseptically to each 9 ml of the mixed medium. After standing over night at room temperature, the basal medium of each flask or tube was inoculated with two drops of the washed ciliate suspension and was incubated at 25 C. In case of incubation in test tubes, the tubes were placed at slanted position of 30 degrees to the horizontal to insure a high surface to volume ratio of the culture medium.

Test proteins: Several proteins (casein, egg protein, zein and gelatin) and protein concentrates (peanut and sweetpotato proteins) were used as testing nitrogen sources. Peanut and sweetpotato proteins were prepared by extraction with 0.05 N NaOH. The crude protein concentrates were precipitated by acetic acid and dried

TABLE I

Composition of basal medium

Ingredient	mcg/ml	Ingredient	mcg/ml
Ca-pantothenate	0.625	$MgSO_4 \cdot 7H_2O$	140
Nicotinamide	0.625	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	62.5
Pyridoxine·HCl	6.25	$MnCl_2 \cdot 4H_2O$	1.25
Pyridoxal	0.625	$ZnCl_2$	0.125
Riboflavin	0.625	$CaCl_2 \cdot H_2O$	30
Thiamine·HCl	6.25	$CuCl_2 \cdot 2H_2O$	3
Folic acid	0.0625	$FeCl_3 \cdot 6H_2O$	0.75
Inositol	0.625	K_2HPO_4	175
Choline chloride	6.25	KH_2PO_4	175
PABA	0.625	Guanine	50
Biotin	0.0625	Adenine sulfate	50
Lipoic acid	0.02	Cytosine	50
Starch or glucose*		Uracil	50

* Different level of supplementation is shown in each experiment.

in vacuo. As suggested by Rosen and Fernell (2), the proteins and amino acids were suspended in distilled water with pH adjusted to 8.2 by NaOH and were refrigerated overnight. After warming to room temperature and mixing with other components, the pH was adjusted to 7.2 before sterilization.

Tween and sterols: For testing the effect of sterols on the growth of the organisms cholesterol and ergosterol were dissolved in Tween 80 by heating in boiling water bath to make 0.06 M solutions. Half ml of the Tween solution was homogenized into each 10 ml medium to give a final concentration of 0.003 M by means of a Teflon homogenizer.

Measurement of growth response: The growth response after incubation was measured by determining microscopically the number of organisms per ml of the test medium. One ml of the pooled sample of triplicate cultures of each test medium was transferred to a test tube containing 0.05 ml of Noland's solution. Aliquot from each pooled sample was counted for the number of ciliates in a Spencer double-cell hemacytometer. All data were obtained from cultures after incubation at 25 C for four days, otherwise specific incubation period will be indicated.

RESULTS

The results of the growth experiments of *T. pyriformis* MC indicate that the ciliates could grow in media with a wide range of pH, where the maximum rate of growth was obtained at pH 7.2–7.4. Under properly aerated condition, the increase of population in the log phase followed approximately a linear function up to the incubation period of 4 days and reached a maximum at 4 to 6 days. Inoculation of variable ciliate population into heterogenous medium could affect the length of incubation period needed to achieve maximum growth; however, in homogenous protein or peptone medium, maximum growth would be obtained always between four to six days of incubation with various inoculated population. Most of the results obtained in the present experiment were secured on the 4th day of incubation,

The growth of ciliates in casein medium with various concentrations of starch or glucose as the energy source supplemented at graded levels is shown in *Figs. 1* or *2* respectively. The results

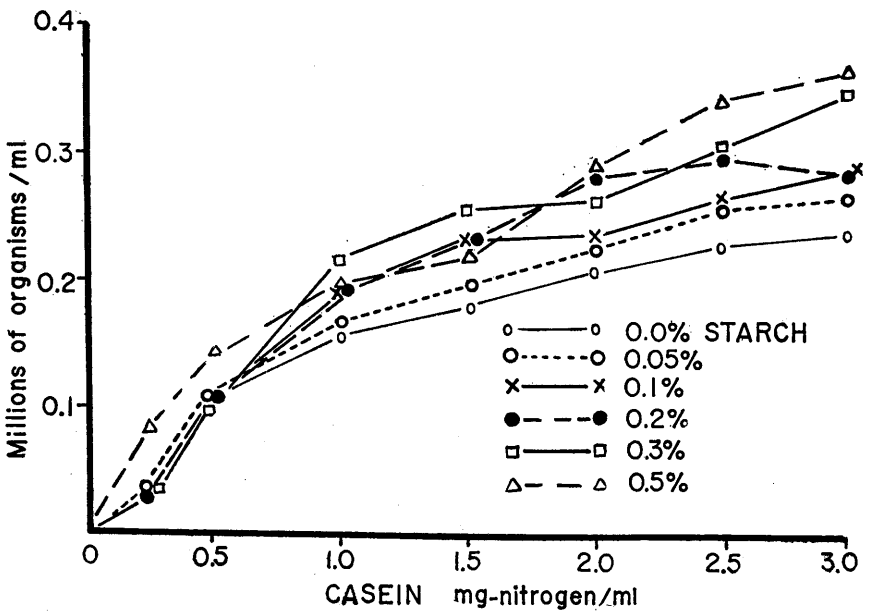

Fig. 1. Effect of starch on growth of *T. pyriformis* MC in casein medium.

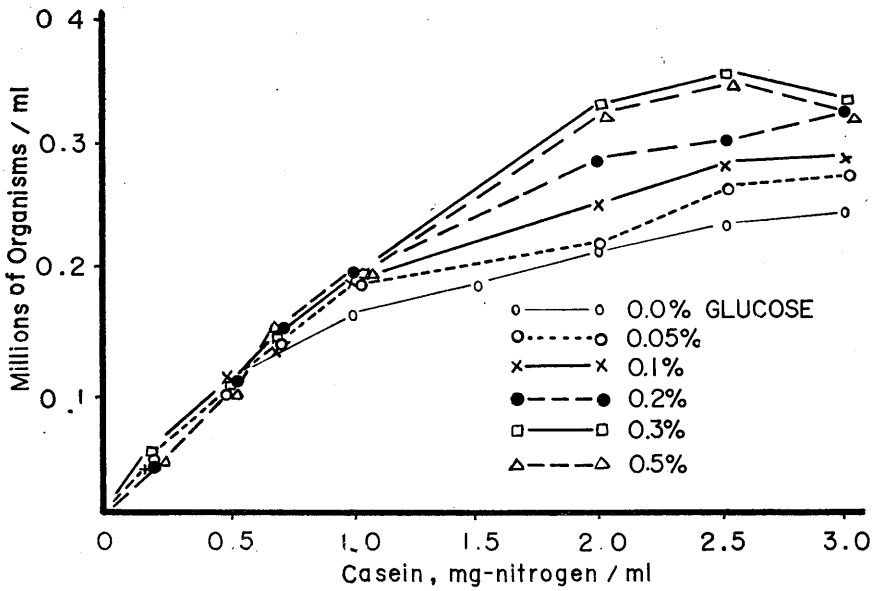

Fig. 2. Effect of glucose on growth of *T. pyriformis* MC in casein medium.

indicate that, at the medium of higher nitrogen contents, supplementation with either glucose or starch gave higher growth response than without carbohydrate supplementation. In media of lower nitrogen contents (< 1 mg-nitrogen/ml), no significant growth promotion effect was obtained with different levels of glucose or starch supplementation. The increase of growth response was nearly proportional to the nitrogen levels in glucose media. A similar trend of growth was obtained in starch media but much higher variation was shown. When glucose or starch media contained higher levels of nitrogen contents (> 1 mg-nitrogen/ml), better growth responses were obtained as the levels of either glucose or starch supplementation was increased. Nevertheless, when the carbohydrate level was beyond 0.3%, there showed no appreciable improvement in growth response.

Rosen and Fernell (1, 2) and Teunisson (4), using higher concentration of carbohydrate (2%) in casein medium for studies on *T. pyriformis* W, obtained successful results, whereas the present results indicated that the optimal ratio of energy source to protein for strain MC was much lower. It is considered that carbohydrate contents higher than 0.3% would not only be wasteful, but the high osmotic pressure exerted by high concentration would likewise inhibit the utilization of nitrogen source by the ciliates.

The growth of ciliates in different protein media with glucose as enegy source is shown in *Fig. 3.* The results show that the nutritive value of the proteins did not correlate to the growth response. Protein concentrate from peanut gave the best growth response which was unexpectedly higher than that of the casein medium. Both whole egg protein and zein failed to support growth regardless of the level of protein in media. It should be mentioned that these two proteins were insoluble and tended to aggregate during heat sterilization. The heterogeneity of these two media which hampered the availability of the medium nitrogen to the ciliates was believed to be the primary cause of low growth response.

When gelatin supplemented with tryptophan or a complete amino acid mixture (medium D of Kidder and Dewey) (5) was used as the nitrogen source,

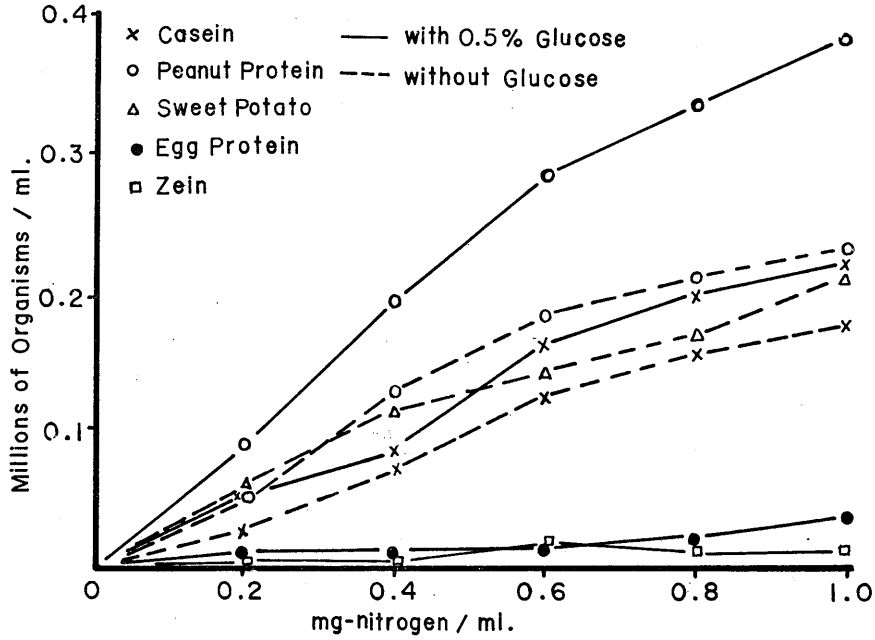

Fig. 3. Growth of *T. pyriformis* MC in media of different proteins.

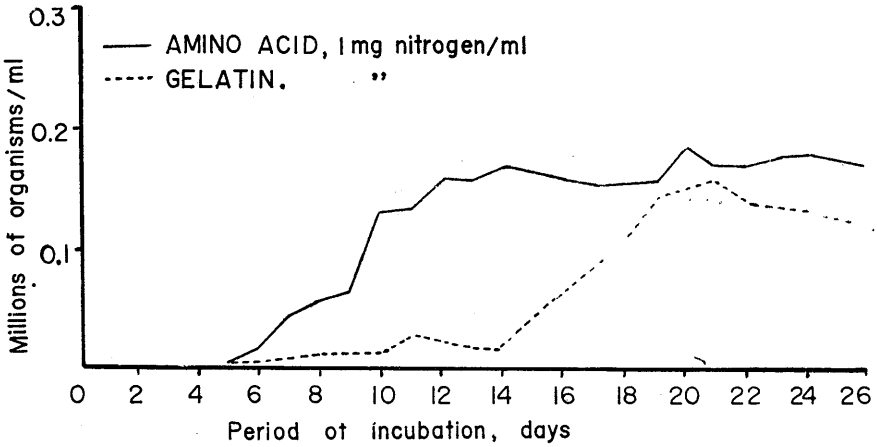

Fig. 4. Growth of *T. pyriformis* MC in amino acid and gelatin media.

unsuccessful result with very small increase of population was obtained after four days of incubation (*Fig. 4*). However, with prolonged incubation, growth could be observed after two weeks. The delayed growth in these media was considered to be due to the necessity of some extra amount of folic and pyridoxal for body protein synthesis as suggegted by Singer (7). However, medium containing high levels of folic acid (10 mcg/ml) and pyridoxal (6 mcg/ml) was tested in this laboratory and unpublished data indicate that the delayed growth response could not be overcome.

The effect of Tween 80 and sterols on growth is shown in TABLE II. Small amount of either Tween alone or Tween

Effect of Tween 80 and sterols on growth
(Millions of organisms/ml)

Protein (Casein) mg/N/ml	Water blank (Control)	Sterol solution	ml of Tween 80 solution added				
			0.1	0.2	0.3	0.4	0.5
0.5	0.088	Tween 80	0.072	0.059	0.059	0.060	0.057
		Cholesterol*	0.065	0.062	0.054	0.040	0.049
		Ergosterol*	0.065	0.061	0.054	0.046	0.042
1.0	0.119	Tween 80	0.102	0.088	0.093	0.078	0.060
		Cholesterol	0.098	0.077	0.071	0.066	0.066
		Ergosterol	0.108	0.083	0.077	0.074	0.046
2.0	0.169	Tween 80	0.121	0.103	0.102	0.091	0.073
		Cholesterol	0.104	0.102	0.087	0.094	0.073
		Ergosterol	0.102	0.098	0.084	0.071	0.067

* 0.06 M solution in Tween 80.

with steroid exerted a significant suppression on the increase of ciliate population. Supplement of 0.1 ml Tween 80 to each 10 ml medium suppressed the growth to about 80% compared with the control. As the supplemented amount of Tween 80 was increased, further decrease of growth response was observed; nevertheless, depression in media with 0.2–0.5 ml of Tween 80 was not significant. With cholesterol or ergosterol supplementation, slightly further depression in growth than Tween alone was obtained. The trend of depression was similar to that of Tween alone.

DISCUSSION

The present study indicates that the growth of *T. pyriformis* MC in chemically defined medium was affected by the type as well as the level of both carbohydrate and nitrogen source; and that in absence of energy source, protein was wastefully utilized for energy purpose, thus restricting the synthesis of organism protetin. Reynolds and Wragg (8) studies the effect of type of carbohydrate on growth and protein synthesis by *T. pyriformis* W and concluded that dextrin supported better growth than glucose. These authors pointed out that carbohydrate level of 1% would be the optimal level. The present findings indicates that no significant difference occurred between glucose and starch, except a more regular rate of population increase was usually shown in glucose medium. These results seem to be in accord with Seaman's suggestion (9), that *T. pyriformis* MC utilized starch in the hydrolyzed form rather than phagotrophic ingestion. The more regular and linear growth response toward nitrogen contents (up to 2 mg-nitrogen/ml) with glucose level at 0.3–0.5% also indicates that glucose is more preferable as energy source than starch in studies where growth response is used as a criterion for comparison.

Rosen and Fernell (1, 2) and Teunisson (4) reported that fairly promising results had been demonstrated on the evaluation of nutritive value of proteins by *T. pyriformis* W and a maximum growth of about one million organisms per ml could be achieved with casein at 0.5–1.0 mg-nitrogen/ml. However, much lower maximum growth (0.3 million/ml) was obtained in the present experiment and the results failed to demonstrate the nutritive value of proteins, although growth res-

ponses obtained were approximately linear to the levels of protein ranged 0-1 mg-nitrogen/ml. The limited growth in media of heterogenous protein suspensions (egg protein and zein) suggests that the rate of proteolytic digestion varied with the homogeneity of the medium as well as the source of proteins. The attempt of using this organism with similar techniques currently employed appears to be impractical for the purpose of protein quality assay.

The abnormal growth of ciliates in gelatin media suggests that the proteolytic enzyme of the ciliates could not hydrolyze gelatin effectively. Thus the slow rate of liberation of amino acids or peptides caused the delayed growth. However, similar interpretation could not be applied to the results obtained from amino acid medium. Yet, there was no direct evidence that the abnormal growth in amino acid medium was attributed to the high osmotic effect, since there was no limitation of growth in protein media with glucose in relatively high concentrations. The osmotic pressure became a primary factor of limited growth in media only with glucose concentrations higher than 2% (w/v) (8). The present test organism might differ from other strains in the requirement of amino acids so that an imbalance or antagonism between amino acids occurred in the present experiments. The delayed growth was also attributable to the effect of metabolic adaptation to different media. A study on the differences in response of related biological systems to the environment will be desirable to acertain the inefficient utilization of medium amino acids by the present ciliates.

It had been demonstrated that 3β-OH sterols stimulated the growth of *T. pyriformis* W and that stigmasterol and cholesterol were shown to increase the phosphate accumulation in *T. pyriformis* W (10, 11). The supplementation of Tween alone has also been known to stimulate the growth of ciliates (5). However, the present finding indicates that both Tween 80 and sterols were growth depressors in short incubation period. The question, whether the unsuccessful growth of the organism was due to the change of physical nature of the media that made the nutrients not readily available to the ciliates or the ciliates needed a period for metabolic adjustment to adapt the environment, remains to be clarified by further investigation on the change of body compositions of the ciliates.

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